

1     **NUCLEIC ACID AND AMINO ACID SEQUENCES OF INFECTIOUS**  
2     **SALMON ANAEMIA VIRUS AND THEIR USES AS VACCINES**

3

4     The present invention relates to a fish vaccine. More  
5     specifically the invention relates to a vaccine to  
6     protect salmon against infectious salmon anaemia virus.

7

8     Infectious salmon anaemia virus (ISAV) causes mortality  
9     of farmed Atlantic salmon. Typically aquaculture  
10    revenue is reduced by over 30%. Accordingly, there is  
11    a need for an effective vaccine against ISAV.

12

13    It is an object of the present invention to provide a  
14    vaccine to protect against ISAV.

15

16    According to the present invention there is provided a  
17    composition containing at least one nucleic acid  
18    sequence and/or at least one amino acid sequence, or a  
19    synthetically prepared analogue thereof or a  
20    substantially homologous sequence, wherein the  
21    composition is derived from or based upon infectious  
22    salmon anaemia virus and wherein at least one of said  
23    nucleotide and/or amino acid sequences does not cause  
24    salmon anaemia and is capable of being used as or to  
25    prepare a vaccine to ISAV.

26

27    A substantially homologous nucleic acid sequence is a  
28    sequence which can be transcribed and/or translated to  
29    provide an amino acid sequence which is substantially

1 homologous to at least a part of a surface antigen  
2 present on ISAV.

3

4 Preferably the substantially homologous amino acid is  
5 at least 70% homologous with a part of a surface  
6 antigen of ISAV which is capable of inducing an immune  
7 response.

8

9 More preferably the substantially homologous amino acid  
10 sequence is at least 80% homologous with a part of a  
11 surface antigen of ISAV and can induce an immune  
12 response.

13

14 Most preferably the substantially homologous amino acid  
15 sequence is at least 90% homologous with a part of a  
16 surface antigen of ISAV and can induce an immune  
17 response.

18

19 Suitably the amino acid sequence is chosen from the  
20 group comprising Sequences ID numbers 2, 4, 6, 7, 8 or  
21 10 as herein described.

22

23 Alternatively the amino acid sequence may comprise at  
24 least one fragment of Sequence ID numbers 2, 4, 6, 7, 8  
25 or 10.

26 Alternatively said amino acid sequence may be truncated  
27 from an amino acid sequence of Sequences ID numbers 2,  
28 4, 6, 7, 8 or 10 as herein described, which can induce  
29 an immune response.

30

31 Preferably the substantially homologous nucleotide  
32 sequence is at least 60% homologous with a part of a  
33 nucleic acid sequence of a surface antigen of ISAV and

1 the translation product thereof is capable of inducing  
2 an immune response.

3

4 Preferably the substantially homologous nucleotide  
5 sequence encodes at least 70% homologous with a part of  
6 a nucleic acid sequence of a surface antigen of ISAV,  
7 the translation product of which is capable of inducing  
8 an immune response.

9

10 More preferably the substantially homologous nucleotide  
11 sequence encodes at least 80% homologous with a part of  
12 a nucleic acid sequence of a surface antigen of ISAV,  
13 the translation product of which is capable of inducing  
14 an immune response.

15

16 Most preferably the substantially homologous nucleotide  
17 sequence is at least 90% homologous to a part of a  
18 nucleic acid sequence of a surface antigen of ISAV, the  
19 translation product of which is capable of inducing an  
20 immune response.

21

22 Suitably the nucleotide sequences are chosen from the  
23 group comprising Sequence ID numbers 1, 3, 5 or 9 as  
24 herein described.

25

26 Alternatively, the invention provides for fragments of  
27 the sequences described in Sequence ID numbers 1, 3, 5  
28 and 9 as herein described and wherein translation  
29 products of said fragments result in the induction of  
30 an immune response.

31

32 Additionally, the sequences may comprise a truncated  
33 form of the sequences given as 1, 3, 5 and 9.

1

2 The nucleotide sequence may be incorporated in a  
3 plasmid.

4

5 The nucleotide sequence may be incorporated in a  
6 suitable expression vector.

7

8 A further aspect of the present invention provides for  
9 the use of a sequence chosen from the group consisting  
10 of Sequence ID numbers 1 to 10, as described in the  
11 present invention in the preparation of a vaccine  
12 and/or therapeutic medicament for the protection of  
13 fish from infection with Infectious Salmon Anaemia  
14 virus.

15

16 Typical nucleic acid sequences are ISA2cd (previously  
17 referred to as p1.38), ISA1mta (previously referred to  
18 as p8.17), ISA3mx (previously referred to as p6.28) and  
19 ISA4ha.

20

21 Preferably the peptide sequences are transcribed and  
22 translated from either one, two or all of the nucleic  
23 acid sequences; ISA2cd, ISA1mta, ISA3mx or ISA4ha and  
24 are incorporated into a vaccination strategy aimed at  
25 inducing an immune response to a surface antigen of  
26 ISAV and thus infectious salmon anaemia virus itself.

27

28 The invention provides the use of nucleic acid  
29 sequences or peptide sequences as defined herein in the  
30 preparation of a vaccine for the protection of fish  
31 against ISAV.

32

1 The invention further provides a vaccine to protect  
2 fish against ISAV wherein the vaccine includes nucleic  
3 acid or peptide sequences as defined herein.

4

5 **Characterisation of the Novel Sequences of the**  
6 **Invention**

7

8 The accompanying figures describe the invention in more  
9 detail, wherein;

10

11 Figure 1 is the nucleotide sequence of ISA2cd,

12

13 Figure 2 is the amino acid sequence which is  
14 obtained from translation of the ISA2cd nucleic  
15 acid sequence listed in Figure 1,

16

17 Figure 3 is the nucleotide sequence of ISA1mta,

18

19 Figure 4 is the amino acid sequence which is  
20 obtained following transcription of the nucleic  
21 acid sequence listed in Figure 3,

22

23 Figure 5 is the exact nucleotide sequence of  
24 ISA3mx,

25

26 Figure 6a is the amino acid sequence (M1) which is  
27 translated from the unspliced nucleic acid  
28 sequence of ISA3mx shown in Figure 5,

29

30 Figure 6b is the amino acid sequence (M2) which is  
31 translated from the spliced nucleic acid sequence  
32 of ISA3mx shown in Figure 5, and

33

1           Figure 6c is the amino acid sequence (M3) which is  
2           translated from the unspliced nucleic acid  
3           sequence of ISA3mx as shown in Figure 5.

4

5       In addition, information detailing the specific  
6       molecular weight (MW) and theoretical isoelectric  
7       focusing points (pI) is given at the foot of the  
8       respective amino acid sequence listings.

9

10      ---The nucleotide and amino acid sequences shown in the  
11      figures are further represented in the accompanying  
12      Patent-In generated sequence listings wherein;

13

14           Sequence ID number 1 is the nucleotide sequence of  
15           ISA2cd, as shown on figure 1,

16

17           Sequence ID Number 2 is the amino acid sequence of  
18           the ISA2cd, as shown in figure 2,

19

20           Sequence ID number 3 is the nucleotide sequence of  
21           ISA1mta, as shown on figure 3,

22

23           Sequence ID number 4 is the amino acid sequence of  
24           ISA1mta, as shown on figure 4,

25

26           Sequence ID number 5 is the nucleotide sequence of  
27           ISA3mx, as shown on figure 5,

28

29           Sequence ID number 6 is the predicted amino acid  
30           sequence of unspliced product of ISA3mx, as shown  
31           in figure 6a,

32

1       Sequence ID number 7 is the predicted amino acid  
2       sequence of spliced ISA3mx, as shown in figure 6b,

3

4       Sequence ID number 8 is the predicted amino acid  
5       sequence of spliced ISA3mx, as shown in figure 6c,

6

7       Sequence ID number 9 is the nucleotide sequence of  
8       ISA4ha, as previously shown in figure 7, and

9

10       Sequence ID number 10 is the amino acid sequence  
11       of ISA4ha, as previously shown in figure 8.

12

13       The genetic sequences shown for ISAlmta and ISA2cd and  
14       the unspliced and spliced genetic sequences for ISA3mx  
15       have been derived from cloned cDNA wherein the cDNA  
16       clones were derived from infectious salmon anaemia  
17       virus (ISAV) genomic material. The cloned material was  
18       sequenced from the 5' end and the 3' end insertion  
19       sites using overlapping amplicons to produce a contig.

20

21       Veracity of the contig was confirmed by Reverse  
22       Transcriptase Polymerase Chain Reaction amplification  
23       (RT-PCR) of appropriate sized amplicons from ISAV  
24       infected salmon tissue and tissue cultures. Such  
25       amplicons were however obtained from uninfected control  
26       material, indicating that the genetic material was of  
27       ISAV origin.

28

29       The open reading frames (ORFs) were completed by rapid  
30       amplification of cDNA ends (RACE) from the incomplete  
31       sequence from virus-infected tissue culture.

32       Corrections were made for the *in vivo* transcribed mRNA

1 that were not apparent from the originally cloned  
2 cDNAs.

3  
4 The ORF from ISA2cd does not have any significant  
5 homology at the nucleotide or amino acid sequence with  
6 previous submissions to databases accessible by BLAST.  
7 However, proteins with similar molecular weights (Mw)  
8 and isoelectric points (pI) include 14 viral proteins  
9 in the Swiss-Prot database such as Hemagglutinin-  
10 -Neuraminidase. -----

11  
12 The ORF from ISA1mta is also without any significant.  
13 homology to previously characterised proteins submitted  
14 to the BLAST searchable databases. However it is of  
15 interest that it has molecular weight and isoelectric  
16 point characteristics (68-69 kDa and pI 8.2) that are  
17 nearly identical to one of the most predominant viral  
18 proteins identified by two dimensional electrophoresis.  
19 The protein appears to be integrally associated with  
20 the membranes of the ISAV infected tissue cultures. If  
21 the ORF yields such a protein it would be considered  
22 valuable in any vaccination strategy to reduce the  
23 level of ISAV infection in any salmonoid species.

24  
25 Further, in the sequences shown for ISA3mx, the  
26 unspliced ORF (the basis for predicted amino acid  
27 sequence M1) does not have any significant homology at  
28 the nucleotide or amino acid sequence level with the  
29 previous submission to databases accessible by BLAST.  
30 However, proteins with similar molecular weights and  
31 isoelectric focusing points include several viral coat  
32 and envelope proteins listed in the Swiss-Prot  
33 database. Both the predicted M1 and M2 proteins



1 (obtained from ORF's following splicing of the  
2 nucleotide sequence) are predicted to be membrane  
3 associated proteins and if the ORFs encoded by ISA3mx  
4 yield such proteins it would be considered valuable in  
5 any vaccination strategy to reduce the level of ISAV  
6 infection in any salmonid species.

7  
8 The predicted protein translation of M3 (shown in  
9 figure 6c and accompanying sequence listing) shows  
10 homology to a paromyxovirus fusion protein associated  
11 with the cell membrane and thought to be involved in  
12 cell adhesion. In view of this exhibited homology, M3  
13 is potentially valuable in any vaccination strategy  
14 aimed at reducing the level of ISAV infection in any  
15 salmonid species.

16  
17 The further sequence relating to ISA4ha nucleotide  
18 sequence was obtained by means of the following  
19 procedure. The ISA4ha protein was detected by  
20 polyclonal antibodies following hybridisation. The  
21 protein is found to occur in two alternative forms.  
22 These two alternative forms are of different sizes, and  
23 can be seen where the proteins are cultured on  
24 different cell lines, for example shc and chse.

25  
26 As these two alternate forms were both detectable by  
27 antibody and varied in size depending on how it was  
28 grown, the protein is potentially a good candidate for  
29 virulence.

30  
31 The protein was isolated and sequenced, resulting in a  
32 24 amino acid fragment being produced. When this  
33 sequence was submitted, to BLAST searchable databases,

1 it showed similarities to sequences of British and  
2 Norwegian strains of ISAV.

3

4 Subsequently, primers were designed based on the amino  
5 acid sequence obtained, along with reference to the  
6 sequences known for the similar British and Norwegian  
7 strains.

8

9 The primers were then subsequently used in polymerase  
10 chain reaction to amplify the relevant DNA fragment,  
11 which was subsequently sequenced and translated into  
12 amino acid coding.

13

14 The open reading frame listings obtained in the present  
15 invention, have particular commercial value for the  
16 following reasons:

17

18 1. There is sufficient reason to believe that the  
19 nucleotide corresponding amino acid sequences are  
20 of ISAV origin. Therefore, their incorporation  
21 into nucleic acid vaccines may have an impact on  
22 the reduction of mortality of farmed Atlantic  
23 salmon caused by ISAV which as previously stated,  
24 can typically reduce aquaculture revenues by over  
25 30%.

26 2. Characterisation of the gene product will lead to  
27 the identification of key elements in the  
28 pathogenesis of infection and to the design of  
29 more accurate diagnostic tests which will also aid  
30 in epidemiological studies documenting the  
31 dissemination of different strains of the disease.

32

1 The nucleotide sequences ISA1mta, ISA2cd, ISA3mx,  
2 ISA4ha and associated derivatives thereof when  
3 translated into protein sequences being composed of  
4 either identical or equivalent amino acids, should  
5 induce a response by the hosts immune system. This  
6 principle can be further expanded to use these proteins  
7 in diagnostics tests and vaccination procedures.